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14. ABSTRACT
This study aims to conduct follow-up longitudinal cognitive evaluations on a sub-sample of 100 Time 3 Ft. Devens Cohort veterans, most of whom were last evaluated in-person for cognitive functioning and with neuroimaging in the mid-1990s, to compare objective measurement of reported decline; and to determine cross-sectional blood and neuroimaging biomarkers (blood and structural volumetrics will also include longitudinal analyses) at 25+ years post deployment to the Gulf region, that may be consistent with cognitive outcomes and presumed pathobiological mechanisms (oxidative stress, ROS) of GWI. These data will evaluate the utility of previously unavailable blood and neuroimaging markers of oxidative stress, to devise a new diagnostic test for GWI in subgroups of GW veterans (TBI and OP exposed), and to provide a potential objective biomarker of treatment efficacy in clinical trials.

15. SUBJECT TERMS Gulf War Illness, central nervous system, biomarkers, glutathione, MR Spectroscopy, Cognition, oxidative stress markers

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1. Introduction:

Background and Purpose: One of the earliest and longest running studies of Gulf War veteran's (GWV) health was conducted with the Ft. Devens, MA army cohort (FDC) from the VA Boston Healthcare System (VABHS). The cohort was first surveyed within 5 days of their return and multiple cross sectional survey and in-person data provided some of the earliest cognitive, neuroimaging and environmental exposure outcomes since the 1990s. These findings included early documentation of the most common health symptoms, cognitive decrements in environmentally exposed GWV, and differences in structural neuroimaging, including lower white matter volumes (Proctor et al., 1998; White et al., 2001; Heaton et al., 2007). The FDC has been followed with longitudinal health surveys, and we are completing a resurvey and biomarker study in which 50% of prior surveyed individuals have responded. This most recent resurvey is providing valuable information pertaining to longitudinal health symptoms and the trajectory of health symptoms over time. Additionally, we are able to use this longitudinal self-report data to monitor CDC and Kansas GWI criteria over time. However, these data are self-report and only a small portion of individuals are being seen for cross-sectional analyses of proteins in the blood (GW100046). Since subsamples of the FDC took part in cognitive and neuroimaging studies between 1994 and 1996, we are now extending these studies by reassessing neurocognitive and neuroimaging status to more fully capitalize on the longitudinal nature of this cohort and the recent findings of oxidative stress markers in GWI.

Scope: The overarching objective of this work study is to build on previous longitudinal studies to gain a better understanding of Gulf War Illness and to devise targeted treatment strategies. This study aims to conduct follow-up longitudinal cognitive evaluations on a sub-sample of 100 Time 3 FDC veterans, most of whom were last evaluated in-person for cognitive functioning and with neuroimaging in the mid-1990s, to compare objective measurement of reported decline; and to determine cross-sectional blood and neuroimaging biomarkers (blood and structural volumetrics will also include longitudinal analyses) at 25+ years post deployment to the Gulf region, that may be consistent with cognitive outcomes and presumed pathobiological mechanisms (oxidative stress, ROS) of GWI. These data will evaluate the utility of previously unavailable blood and neuroimaging markers of oxidative stress, to devise a new diagnostic test for GWI in subgroups of GWV (TBI and OP exposed), and to provide a potential objective biomarker of treatment efficacy in clinical trials.

2. Key Words: Gulf War Illness, central nervous system, biomarkers, glutathione, MR Spectroscopy, Cognition, oxidative stress

3. Accomplishments:

- What were the major goals of the project?
    - The major goals of the project as stated in the approved SOW for year1 is listed in the table below. Specifically, during year 1, the primary goals were to obtain Boston VA and Boston University IRB approvals and DOD HRPO approvals. Planning for study protocols for brain imaging, cognitive testing and oxidative stress blood markers was also a goal of this year. Milestones/target dates for important activities or phases of these dates are listed in the table and actual completion dates are listed below.

Tasks	Timeline
Task 1. Obtain necessary authorization prior to initiation of human subjects	**Months**
1a. Obtain Institutional Review Board (IRB) approval for research sites at VA Boston (VABHS), Boston University Medical Campus (BUMC), and Nova University (NSU) for protocols	1-4
1b. Obtain DOD Human subjects Research Protections Office (HRPO) approvals	5-7
1c. Complete hiring of necessary staff and ensure all mandatory IRB research related trainings are completed by all staff members	1-8
Task 2. Preparation and Training for Clinical Study Procedures	**Months**
2a. Obtain Time 3 cognitive and MRI neuroimaging data for longitudinal analyses and participant contact information from the Ft. Devens cohort (FDC) study through the share drive at VABHS.	1-2
2b. Develop manuals for neuropsychological testing protocol, structural MRI and Magnetic Resonance Spectroscopy (MRS) of glutathione oxidative stress marker (GSH) protocols and blood specimen collection protocols for several oxidative stress markers.	1-6
2c. Train researchers and staff on cognitive, neuroimaging and phlebotomy protocols and quality control measures.	6-9
Task 3. Screening, recruitment and longitudinal assessment of FDC Gulf War veterans	**Months**
3a. Obtain informed consent from potentially eligible GW veterans	9-36
3b. Assess 150 FDC veterans and obtain demographics, medical history, self-report questionnaires and neuropsychological testing for planned longitudinal analyses.	9-36
3c. Perform brain GSH MR Spectroscopy and structural MRI imaging and blood draw for oxidative stress markers from 100 Gulf War veterans for cross-sectional study.	9-36
Task. 4. Data Cleaning and MRI/MRS image Post-processing	**Months**
4a. Post-process MRI/MRS neuroimaging data for data analysis.	18-40
4b. Score neuropsychological test data and upload summary data to VA Share drive for entry, cleaning and analyses.	18-38
4c. Ship blood samples to Nova University for analysis of GSH oxidative stress markers including (HNE, 8-iso-PGF2α).	18-36
4d. Perform analyses of plasma oxidative stress markers.	18-40
Task. 5. Merge Data and Perform Interim Data analyses	**Months**
5a. Data entry of all questionnaires, cognitive evaluations and quality control measures will be ongoing.	18-42
5b. Interim Statistical analyses of data obtained from cognitive evaluations, blood markers, neuroimaging and questionnaire data will	18-42

be performed periodically.	
5c. Annual reports of progress will be written.	18-36
Task 6. Perform Final Data Analysis and Prepare Manuscripts for Publication (months 42-48)	**Months**
6a. Perform cross-sectional analyses comparing central and peripheral markers of oxidative stress in brain MRS (GSH) and plasma (HNE, 8-iso-PGF2α) compared with cognitive functioning and health symptom report in FDC veterans.	42-45
6b. Perform longitudinal analyses of structural MRI imaging, cognitive, and health symptom outcomes from Time 3 and Time 6 in FDC veterans.	42-46
6c. Write final study report	47-48
6d. Present findings at scientific meetings	42-48
6e. Prepare manuscripts for submission for cross-sectional and longitudinal studies.	42-48

- What was accomplished under these goals?
- Task 1:
    - We obtained necessary authorization prior to initiation of study (IRB approvals, DoD HRPO approvals).
    - We completed the hiring of staff and ensured that all mandatory trainings are now completed.
- Task 2:
    - We have obtained Time 3 cognitive and MRI neuroimaging data for the longitudinal analysis and participant contact information form the Ft. Devens cohort (FDC) study and Treatment Seeking Cohort (TSC) through the share drive at VA Boston Health Care System (VABHS).
    - We developed manuals for the neuropsychological testing protocol as well as structural MRI and MRS of glutathione oxidative stress marker (GSH) protocols and blood specimen collection protocols for oxidative stress markers.
    - We have trained the researchers and staff on cognitive neuroimaging and phlebotomy protocols and questionnaires.

- How were the results disseminated to communities of interest?
    - Our results from analyzing data from the Ft. Devens cohort specifically related to mTBI were presented at two meetings of the International Neuropsychological Society meetings and two recently published papers (see Appendix).

- What do you plan to do during the next reporting period to accomplish the goals?
    - We plan to recruit 50 study participants by the next reporting period and present the preliminary results at appropriate National and International meetings.
    - We plan to submit other manuscripts of preliminary results from the GWI case-control and mTBI exposed vs non-exposed groups and the cognitive, MR Spectroscopy glutathione brain imaging and blood oxidative stress markers during the next reporting period.

4. Impact:

- What was the impact on the development of the principal discipline(s) of the project?
    - Gulf War Illness (GWI) can have a dramatic impact on the lives and well-being of GW veterans who experience chronic and often debilitating symptoms. The results of this study will help address a critical knowledge gap regarding the nature of continued cognitive symptoms and other chronic health effects of GWI.
    - This project will distinguish itself by examining the nature and trajectory of symptoms by adding objective markers of longitudinal neurocognitive decline, traditional structural MRI imaging and cutting-edge MRS brain imaging techniques of oxidative stress markers (glutathione) compared with plasma oxidative stress markers. When combined with the prior rich 20+ year longitudinal data from the Ft. Devens and Treatment-seeking cohorts, this provides an unprecedented opportunity to further characterize objective biomarkers of illness in a well-characterized cohort of GW veterans.
    - Defects in modulation of oxidative stress may well predispose individuals to damage from reactive oxygen species (ROS) from environmental exposures, TBI or other sources that could potentially be used as a diagnostic marker of illness.
    - This analysis also offers an opportunity to determine whether a given therapeutic strategy such as antioxidants including co-enzyme Q-10 or quercetin supplementation in subgroups with low brain glutathione levels may be chosen as a treatment option to improve a susceptible individual's ability to modulate oxidative stress, reduce accelerated aging and improve the symptoms of GWI utilizing a personalized medicine approach.

- What was the impact on other disciplines?
    - A major advantage of work with the Ft. Devens cohort showing mTBI to be related to rates of GWI in our two most recent papers suggests that the results of this study with oxidative stress glutathione markers may be relevant not only to GWI but also to other veteran and civilian groups with mTBI and neurotoxicant exposures as part of a multiple-hit hypothesis.
    - Blood and neuroimaging-based biomarkers of GWI provide an effective way to enhance its management:
        - It can be used as a diagnostic and prognostic tool with the ability to provide information about rate of disease progression.
        - It would help in identification of novel and effective treatments for multiple disorders and environmental exposure groups (i.e. pesticides, nerve agents).
        - It could be used for monitoring therapeutic efficacy for multiple disorders
        - It could provide a cost-effective option for recruitment into clinical trials

- What was the impact on technology transfer?
    - The biomarker that we hope to develop will be cost effective, available, and do not need expensive technicians if we can identify an oxidative stress biomarker in blood that be correlated with MR spectroscopy brain imaging markers that we will also collect and analyze.

- What was the impact on society beyond science and technology?
    - Our blood and brain imaging biomarkers should improve the quality of life for the veterans of the GW who have GW illness because:
        - Our biomarkers can provide objective evidence thereby validating the chronic health symptoms of ill GW veterans.
        - Our biomarkers should lead to studies to develop treatment of brain injury that may lead to improvement of their clinical condition.

5. Changes for approach and reasons for change:

- Changes:
- None

- Problems:
- No problems

- Actual or anticipated problems or delays and actions or plans to resolve them techniques
    - We have been slightly delayed in getting subject recruitment started but we recently had a team meeting in Boston to finalize our plans for recruitment and our study coordinator began contacting FDC veterans to start subject recruitment for the study.

- Changes that had significant impact on expenditures
    - None
- Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:
    - Significant changes in use or care of human subjects: None
    - Significant changes in use or care of vertebrate animals: None
    - Significant changes in use of biohazards, and/or select agents: None

6. Products:

- **Publications, conference papers, and presentations**
    - **Journal Publications**

- Yee MK, Janulewicz PA, Seichepine DR, Sullivan KA, Proctor SP, Krengel MH. Multiple Mild Traumatic Brain Injuries Are Associated with Increased Rates of Health Symptoms and Gulf War Illness in a Cohort of 1990-1991 Gulf War Veterans. Brain Sci. 2017 Jul 9;7(7). pii: E79. doi: 10.3390/brainsci7070079. See Appendix 1.
- Yee, M., Seichepine, D., Januelwicz Lloyd P., Sullivan, K., Proctor, SP & Krengel, M. Traumatic brain injury, health and rate of chronic multisymptom illness in veterans from the 1990-1991 Gulf War. Journal of Head Trauma Rehabilitation. 2016 Sep-Oct;31(5):320-8. doi: 10.1097/HTR.0000000000000173.

    - **Books or other non-periodicals, one-time publications**
        - None

- **Other publications, conference papers, and presentations**
  - Two posters were presented during the annual International Neuropsychological Society Meeting in Washington, DC entitled:

  - Krengel MH, Yee M, Nolan T, Janulewicz Lloyd PA, Sullivan K & Seichepine DR. Multiple Self-Reported Exposures to Mild Traumatic Brain Injury and Neurotoxicants Predict Current Total Health Symptoms in a Cohort of 1990-1991 Gulf War Veterans. Journal of International Neuropsychological Society. Supplement 1, March 2016.

  - Yee, M., Seichepine DR, Nolan T, Janulewicz Lloyd PA, Sullivan K & Krengel MH. Multiple Self-Reported Brain Injuries are Associated with Increased Health Symptoms in a Cohort of 1990-1991 Gulf War Veterans. Journal of International Neuropsychological Society. Supplement 1, March 2016.

- **Other products**

- **Website(s) or other Internet site(s)**
  - **None**

- **Technologies or techniques**
  - **None**

- **Other products**
  - **None**

7. Participants and other Participating Organizations

Site 1: VA Boston Health Care System (VABHS) (BUMC) 150 S. Huntington Avenue
Boston, MA 02130
Initiating PI: Maxine Krengel, PhD

Site 2: Boston University Medical Campus
715 Albany Street
Boston, MA 02118
Partnering PI: Kimberly Sullivan, PhD
Co-Investigator: Carole Palumbo, PhD
Co-Investigator: Ronald Killiany, PhD

Site 3: Nova Southeastern University (NSU)
3200 South University Drive
Fort Lauderdale, Florida 33328-2018
Co-Investigator: Richard C. Deth, PhD
Collaborator: Nancy Klimas, MD

Study Sites Responsibilities

Site 1: Dr. Krengel and her VABHS team will be responsible for recruiting FDC study participants and conducting cognitive evaluations and phlebotomy to send to NOVA investigators. Specifically, she will oversee the recruitment and blood draws/cognitive evaluations of FDC study participants and the processing of plasma samples that will be shared for the proposed study. Dr. Krengel will also oversee the experimental design, data analysis and

interpretation and presentation of study results in collaboration with Dr. Sullivan and the other study investigators. **Tasks 1-6**

Site 2: Dr. Sullivan and her BUMC team will be responsible for performing the MRS/MRI imaging protocols and post-processing the imaging data for cross-sectional (MRS) and longitudinal analyses (structural MRI). Specifically, she will oversee the imaging acquisition and post-processing protocols in collaboration with Drs. Killiany and Palumbo. Dr. Sullivan will also assist with the experimental design, data analysis and interpretation and presentation of study results in collaboration with Dr. Krengel and the other study investigators. **Tasks 1-6**

Site 3: Dr. Deth and Klimas will be responsible for receiving the plasma samples from the Boston site and performing oxidative stress marker analyses for GSH, HNE and 8-iso-PGF2α for 100 blood samples (50 GWI, 50 controls). Dr. Deth will also assist with the experimental design, interpretation of data, report and manuscript writing and presentation of results at scientific meetings. **Tasks 1, 2, 4, 5, 6**

Reporting Requirements:
None